Acta Crystallographica Section D
**Biological Crystallography**
ISSN 0907-4449

# Co-crystallization of *Staphylococcus aureus* peptide deformylase (PDF) with potent inhibitors

**Melissa S. Harris,[a]* Jeffrey H. Bock,[b] Gil Choi,[c] Joyce S. Cialdella,[b] Kimberly A. Curry,[b] Martin R. Deibel Jr,[d] E. Jon Jacobsen,[a] Vincent P. Marshall,[b] Robert W. Murray Jr,[b] Anne F. Vosters,[d] Cindy L. Wolfe,[d] Anthony W. Yem[a,d] and Eric T. Baldwin[a]**

[a]Structural, Analytical and Medicinal Chemistry, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49007, USA, [b]Infectious Diseases Biology, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49007, USA, [c]Molecular Biology, Human Genome Sciences Inc., Rockville MD 20850, USA, and [d]Protein Science, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49007, USA

Correspondence e-mail: melissa.s.harris@pharmacia.com

Received 18 June 2002
Accepted 2 September 2002

In bacteria the biosynthesis of all nascent polypeptides begins with *N*-formylmethionine. The post-translational removal of the *N*-formyl group is carried out by peptide deformylase (PDF). Processing of the *N*-formyl group from critical bacterial proteins is required for cell survival. This formylation/deformylation cycle is unique to eubacteria and is not utilized in eucaryotic cytosolic protein biosynthesis. Thus, inhibition of PDF would halt bacterial growth, spare host cell-function, and would be a novel mechanism for a new class of antibiotic. Diffraction-quality Se-met crystals of *S. aureus* PDF were prepared that belong to space group $C222_1$ with unit cell parameters of $a = 94.1$ $b = 121.9$ $c = 47.6$ Å. Multiple anomalous dispersion data were collected at the Advanced Photon Source 17-ID beamline and used to solve the PDF structure to 1.9 Å resolution. Crystals were also prepared with three PDF inhibitors: thiorphan, actinonin and PNU-172550. The thiorphan and actinonin co-crystals belong to space group $C222_1$ with similar unit-cell dimensions. Repeated attempts to generate a complex structure of PDF with PNU-172550 from the orthorhombic space group were unsuccessful. Crystallization screening identified an alternate *C*2 crystal form with unit-cell dimensions of $a = 93.4$ $b = 42.5$ $c = 104.1$ Å, $\beta = 93^\circ$.

## 1. Introduction

Initiation of translation in eubacteria utilizes formylmethionine-charged −tRNA for the ribosome-mediated biosynthesis of the first peptide bond. As a result, all nascent polypeptides are synthesized with *N*-formyl methionine at the N-terminus (Meinnel & Blanquet, 1995). The formyl group is removed by peptide deformylase (PDF) during elongation or shortly after completion of polypeptide biosynthesis (Adams, 1968; Ball & Kaesberg, 1973; Livingston & Leder, 1969). Removal of the formyl group is essential to cell survival and it has been shown that deletion of the PDF gene in *Escherichia coli* is lethal (Mazel *et al.*, 1994; Clements *et al.*, 2001). This formylation/deformylation cycle is unique to eubacteria and is not utilized in eukaryotic cytosolic protein biosynthesis. The deformylation activity of PDF is an attractive target for a potential new class of antibiotics. A structure-based drug-design effort was initiated to support the elaboration of specific PDF inhibitors. Ligand-free PDF crystals and three complexes of PDF with potent ligands from the human pathogen *Staphylococcus aureus* were prepared and X-ray diffraction data were collected to beyond 2.0 Å resolution.

## 2. Experimental procedures

### 2.1. Expression and purification

The *S. aureus* peptide deformylase sequence was identified, cloned and purified as previously described (Baldwin *et al.*, 2002). The gene encodes a total of 189 residues, including a C-terminal hexahistadine tag. The molecular weight of the PDF protein is 19 kDa. Protein was provided for crystallization in 25 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl at a protein concentration between 30 and 60 mg ml$^{-1}$. Protein samples were diluted to 30 mg ml$^{-1}$ with matching buffer solution, snap-frozen in liquid nitrogen and stored at 193 K for subsequent crystallization experiments. Selenomethionine-enriched PDF was also expressed and purified for crystallization experiments. Incorporation of SeMet was accomplished through the down-regulation of methionine biosynthesis just prior to induction of PDF expression with isopropyl $\beta$-D-1-thiogalactopyranoside (Benson *et al.*, 1995; Van Duyne *et al.*, 1993).

### 2.2. Ligand-free crystallization

Sparse-matrix crystallization screens of ligand-free PDF were set up in 2 µl hanging

drops at 293 K (Jancarik & Kim, 1991). After 14 d, long thin needle clusters were observed from Hampton Crystal Screen condition No. 6 (30% PEG 4000, 0.2 *M* $MgCl_2$ and 0.1 *M* Tris pH 8.5) (Fig. 1). Grid-screen optimization experiments varying PEG 4000 and $MgCl_2$ concentrations, pH and drop size continued to produce thin rod-shaped clusters. Single rod-shaped crystals were finally achieved by microseeding 8 µl sitting drops at set up with a $10^{-3}$ diluted seed stock using a cat's whisker. Seed-stock dilutions were prepared by crushing needle clusters in stabilization solution (25% PEG 4000, 0.2 *M* $MgCl_2$ and 0.05 *M* Tris pH 8.5) using the seed bead kit (Hampton Research). A crystal measuring 0.22 × 0.20 × 0.60 mm grew from 20% PEG 4000, 0.1 *M* $MgCl_2$ and 0.05 *M* Tris pH 8.5 (Fig. 1). Crystals were cryoprotected over a 2 h time period by slowly adding increasing cryosolution (25% PEG 4000, 0.1 *M* $MgCl_2$, 0.05 *M* Tris pH 8.5 and 25% glycerol) directly to the drops. Crystals were cryocooled in liquid nitrogen and stored for cryogenic data collection. SeMet-enriched protein crystals were also prepared by the method described.

### 2.3. Co-crystallization with potent inhibitors

Three inhibitors were selected for co-crystallization experiments with *S. aureus* PDF (Fig. 2). Two previously reported PDF inhibitors were chosen: actinonin ($IC_{50}$ = 3 n*M*) and thiorphan ($IC_{50}$ = 12 µ*M*) (Chen *et al.*, 2000; Clements *et al.*, 2001; Meinnel *et al.*, 1999). The third inhibitor, PNU-172550, with $IC_{50}$ = 27 n*M*, was discovered during high-throughput screening of a portion of the Pharmacia compound collection using the Vibrio aminopeptidase assay previously described in Baldwin *et al.* (2002).

PDF protein was mixed with 5 m*M* thiorphan, 2 m*M* actinonin or 5 m*M* PNU-172550 and incubated on ice for 1 h. All inhibitor stock solutions were prepared at 100 m*M* in 100% dimethyl sulfoxide. Sitting-drop vapour-diffusion experiments were set up by mixing 4 ul protein and 4 ul well solution containing 17–28% PEG 4000, 0.1 *M* $MgCl_2$ and 0.05 *M* Tris pH 8.5. After one to two weeks, heavy precipitation and needle clusters were observed at the higher PEG 4000 concentrations, but single crystals did not grow spontaneously. Single rod-shaped crystals were achieved with all three ligands as a result of microseeding with a $10^{-4}$ diluted ligand-free seed stock 1 d after setup. Single crystals grew within two weeks (Fig. 2). Crystals were cryopreserved as described for the ligand-free crystals in 25% PEG 4000, 0.1 *M* $MgCl_2$, 0.05 *M* Tris pH 8.5, 25% glycerol and 2–5 m*M* ligand.

Several attempts to obtain a complex structure of PDF and PNU-172550 from the above crystal form proved unsuccessful. Sparse-matrix screening using PDF (15 mg $ml^{-1}$) incubated with 1 m*M* PNU-172550 was initiated to identify a novel crystal form more amenable to PNU-172550 binding. Emerald Biostructures Wizard Screen 1 condition No. 6 (20% PEG 3000 and 0.1 *M* citrate pH 5.5) produced small plate clusters. Larger plate clusters were obtained by lowering the precipitant concentration, setting up larger 8 µl drops and converting to sitting-drop vapour diffusion. Crystals grew to up to 0.2 × 0.1 × 0.03 mm in 5–10 d from 14% PEG 3000 and 0.1 *M* sodium citrate pH 5.5 (Fig. 2). Plates were cryopreserved in 14% PEG 3000, 0.1 *M* sodium citrate pH 5.5, 30% glycerol and 5 m*M* PNU-172550 by slow addition of the cryoprotectant directly to the drop. Individual plates were then teased from the cluster and cryocooled in liquid nitrogen for cryogenic data collection.

### 2.4. Data collection and processing

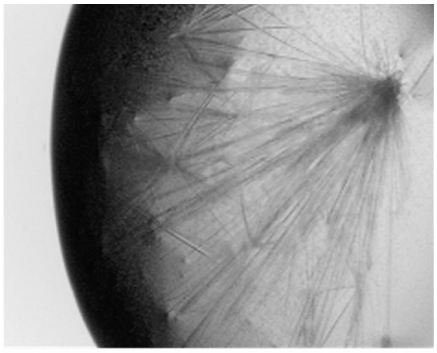
Data were collected using synchrotron radiation at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT), beamline 17-ID at the Advanced Photon Source, Argonne National Laboratory. The ring current varied between 100 and 67 mA and the data sets were collected at 100 K using an Oxford Cryosystems Cryostream. The SeMet data sets were collected at three wavelengths (1.0332, 0.97939, 0.97928 Å) corresponding to the low-energy wavelength, inflection-point wavelength and the peak wavelength about the Se $\kappa$ absorption edge. PDF–inhi-

**Table 1**
Data-collection parameters and preliminary processing results for the low-energy wavelength SeMet data set and each of the three complex data sets.

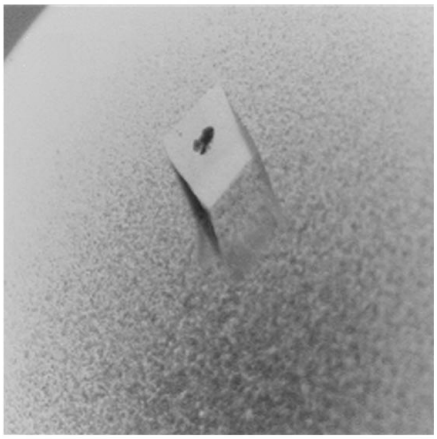
Values in parentheses are for the outermost resolution shell.

| | SeMet (low) | Thiorphan | Actinonin | PHA-172550 |
|---|---|---|---|---|
| Wavelength (Å) | 1.0332 | 1.0000 | 1.0000 | 1.0000 |
| Crystal-to-detector distance (cm) | 15.0 | 15.0 | 12.0 | 14.0 |
| Exposure time (s) | 0.5 | 1.0 | 2.0 | 1.0 |
| No. of frames | 300 | 300 | 120 | 600 |
| Frame width (°) | 0.25 | 0.25 | 1.00 | 0.50 |
| Space group | $C222_1$ | $C222_1$ | $C222_1$ | $C2$ |
| Unit-cell parameters (Å) | | | | |
| $a$ (Å) | 94.1 | 94.1 | 94.1 | 93.4 |
| $b$ (Å) | 121.9 | 121.9 | 121.9 | 42.5 |
| $c$ (Å) | 47.6 | 47.6 | 47.6 | 104.1 |
| $\gamma$ (°) | | | | 93 |
| Resolution range (Å) | 20.0–1.8 | 20.0–1.5 | 20.0–2.0 | 20.0–1.9 |
| No. of observations | 96952 | 70184 | 83619 | 191091 |
| No. of unique reflections | 21188 | 36628 | 17489 | 32247 |
| Completeness (%) | 97.5 | 85.3 (25.8) | 96.7 (77.4) | 99.4 (95.7) |
| $R_{sym}$† (%) | 4.2 (10.7) | 5.1 (20.9) | 3.5 (16.2) | 3.8 (18.9) |
| $\langle I/\sigma(I)\rangle$ | 31.6 (8.0) | 30.0 (1.43) | 30.0 (6.9) | 49.7 (7.0) |
| Mosaicity (°) | 0.53 | 0.573 | 0.473 | 0.59 |

† $R_{sym} = (\sum |I - \langle I\rangle|)/\sum(I)$, where $I$ is the observed intensity and $\langle I\rangle$ is the average intensity of symmetry-related reflections.



(*a*)



(*b*)

**Figure 1**
(*a*) Needle clusters of SeMet PDF were observed after two weeks from Hampton Crystal Screen condition No. 6. (*b*) Single rod-shaped crystals measuring 0.35 × 0.35 × 0.70 mm were finally achieved by microseeding.

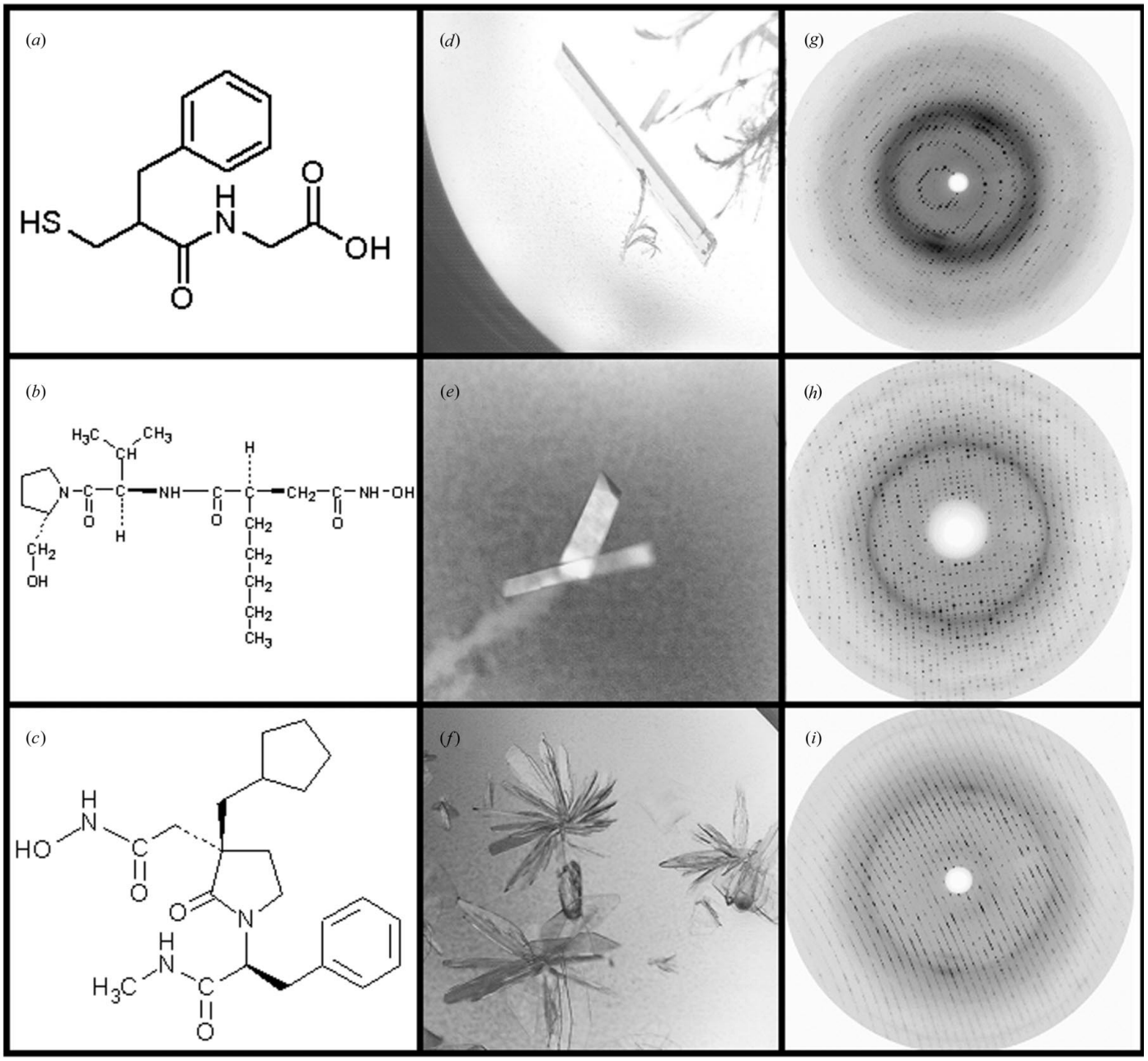



**Figure 2**
Three ligands were selected for co-crystallization experiments with PDF: (*a*) thiorphan, (*b*) actinonin and (*c*) PNU-172550. Co-crystals of PDF complexed with the three inhibitors are shown in (*d*) for thiorphan (0.08 × 0.08 × 0.37 mm), (*e*) for actinonin (0.15 × 0.10 × 0.53 mm) and (*f*) for PNU-172550 (0.2 × 0.10 × 0.03 mm). Single diffraction images from each co-crystal are shown in (*g*), (*h*) and (*i*), respectively.

bitor complex data sets were collected at 1.000 Å. The SeMet-enriched PDF and thiorphan co-crystal data were collected using a Brüker CCD detector mounted upon a $\kappa$ goniometer and the diffraction data were acquired and processed with *SAINT* (Siemens, 1993). The actinonin and PNU-172550 data sets were collected using a MAR CCD detector with *marccd* data-acquisition software (MAR, USA; M. Blum, unpublished work). Two-dimensional images were integrated and scaled with *HKL*2000 (Otwinowski & Minor, 1997). Data-collection parameters and processing statistics for the low-energy wavelength SeMet PDF data set and each of the three ligand–complex data sets are shown in Table 1.

## 3. Results and discussion

Analysis of data from the ligand-free SeMet PDF crystal indicates that it belongs to space group $C222_1$, with unit-cell parameters $a$ = 94.1, $b$ = 121.9, $c$ = 47.6 Å. The crystal diffracts to 1.9 Å resolution and the ligand-free crystal structure was successfully phased using the multiple anomalous dispersion data; a detailed description of this structure is reported elsewhere (PDB code 1lmh; Baldwin *et al.*, 2002). Generation of single PDF–inhibitor co-crystals using the cross-microseeding method with the ligand-free seeds proved very successful. The thiorphan and actinonin co-crystals also belong to space group $C222_1$, with similar unit-cell parameters. The co-crystals diffract strongly to 1.8 and 2.0 Å. There is one molecule per asymmetric unit in the $C222_1$

crystals and the specific volume $V_M$ is 3.585 Å$^3$ Da$^{-1}$ with a solvent content of 65.7% (Matthews, 1968). Difference Fourier maps using data from the $C222_1$ co-crystals confirmed the presence of thiorphan and actinonin in the PDF active site. Attempts to obtain a complex structure of PDF with PNU-172550 in the orthorhombic space group failed repeatedly. This was surprising given the inhibitor's potent binding, good solubility and our observation of another hydroxamate inhibitor, actinonin, in a structure belonging to the $C222_1$ space group. Analysis of the 1.9 Å resolution data generated from the PDF–PNU-172550 plate co-crystal reveals it to belong to space group $C2$, with unit-cell parameters $a$ = 93.4, $b$ = 42.5, $c$ = 104.1 Å, $\gamma = 93^\circ$. There are two molecules per asymmetric unit in the $C2$ co-crystal, with a specific volume of 2.69 Å$^3$ Da$^{-1}$ and a solvent content of 54.3% (Matthews, 1968). The complex structure, solved by molecular replacement, reveals the binding mode of PNU-172550 and will be described elsewhere.

These crystallization experiments have established routine methods for the preparation of *S. aureus* PDF crystals with inhibitors that diffract to beyond 2.0 Å resolution. We have found that micro-seeding greatly increases both the speed and success rate for PDF co-crystallization. Finally, we note that not all validated inhibitors will co-crystallize in the $C222_1$ crystal form. However, novel crystallization conditions can be obtained that produce crystal forms, such as $C2$, that are more favourable for inhibitor binding.

The authors wish to thank June Lull for carrying out amino-acid analysis, Gloria Pokorski for solution preparations, and W. Rodney Matthews and Eric T. Lund for mass-spectroscopy analysis. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy Sciences, Office of Science. Data were collected at beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through the IIT's Center for Synchrotron Radiation Research and Instrumentation.

## References

Adams, J. M. (1968). *J. Mol. Biol.* **33**, 571–589.
Baldwin, E. T., Harris, M. S., Yem, A. W., Wolfe, C. L., Vosters, A. F., Curry, K. A., Murray, R. W., Bock, J. H., Marshall, V. P., Cialdella, J. A., Choi, G. & Deibel, M. R. Jr (2002). *J. Biol. Chem.* **277**, 31163–31171.
Ball, L. A. & Kaesberg, P. (1973). *J. Mol. Biol.* **79**, 531–537.
Benson, T. E., Filman, D. J., Walsh, C. T. & Hogle, J. M. (1995). *Nature Struct. Biol.* **2**, 644–653.
Chen, D. Z., Patel, D. V., Hackbarth, C. J., Wang, W., Dreyer, G., Young, D. C., Margolis, P. S., Wu, C., Ni, Z. J., Trias, J., White, R. J. & Yuan, Z. Y. (2000). *Biochemistry*, **39**, 1256–1262.
Clements, J. M., Beckett, R. P., Brown, A., Catlin, G., Lobell, M., Palan, S., Thomas, W., Whittaker, M., Wood, S., Salama, S., Baker, P. J., Rodgers, H. F., Barynin, V., Rice, D. W. & Hunter, M. G. (2001). *Antimicrob. Agents Chemother.* **45**, 563–570.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Livingston, D. M. & Leder, P. (1969). *Biochemistry*, **8**, 435–443.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mazel, D., Pochet, S. & Marliere, P. (1994). *EMBO J.* **13**, 914–923.
Meinnel, T. & Blanquet, S. (1995). *J.Bacteriol.* **177**, 1883–1887.
Meinnel, T., Patiny, L., Ragusa, S. & Blanquet, S. (1999). *Biochemistry*, **38**, 4287–4295.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Siemens (1993). *SAINT Software Reference Manual*, Pub. No. 269–014200, Siemens Industrial Automation Inc., Madison, WI, USA.
Van Duyne, G. D., Standaert, R. F., Karplus, P. A., Schreiber, S. L. & Clardy, J. (1993). *J. Mol. Biol.* **229**, 105–124.